

Fig. 1. Detecting protein-protein interactions on glass slides. (A) Slide probed with 0.5 ug/mL BODIPY-FL-IgG. (B) Slide probed with 0.1 ug/mL Cy3-IKbA. (C) Slide probed with 0.5 ug/mL Cy5-FKBP12 + 100 nM rapamycin. (D) Slide probed with 0.5 ug/mL Cy5-FKBP12 (no rapamycin). (E) Slide probed with 0.5 ug/mL BODIPY-FL-IgG + 0.1 ug/mL Cy3- IKbA + 0.5 ug/mL Cy5-FKBP12 + 100 nM rapamycin. In all panels, BODIPY-FL, Cy3, and Cy5 fluorescence were false-colored blue, green, and red, respectively.

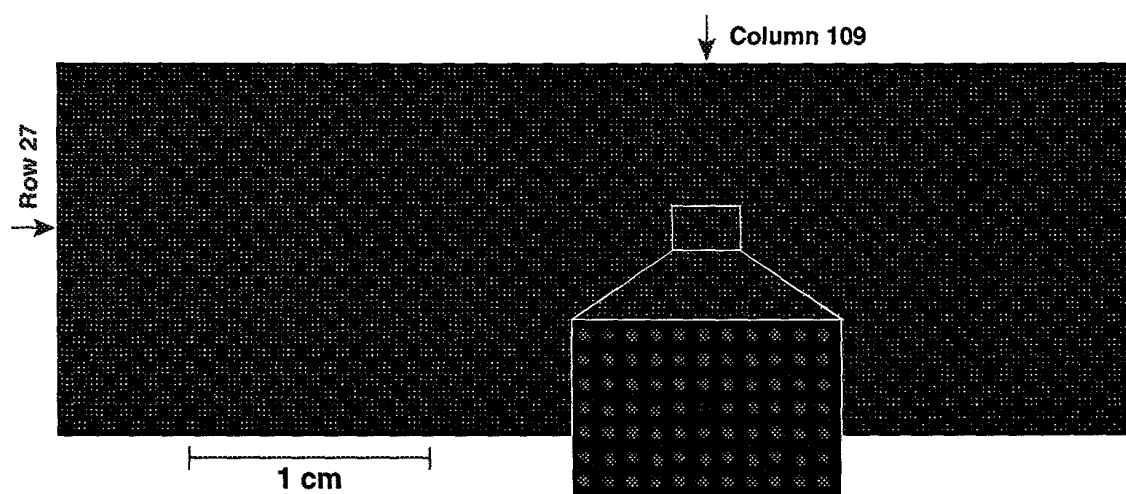


Fig. 2. 10,800 spots on a single slide. Protein G was printed 10,799 times. A single spot of GST-FRB was printed in row 27, column 109. The slide was probed with 0.5 ug/mL BODIPY-FL-IgG + 0.5 ug/mL Cy5-FKBP12 + 100 nM rapamycin. BODIPY-FL and Cy5 fluorescence were false-colored blue and red, respectively.

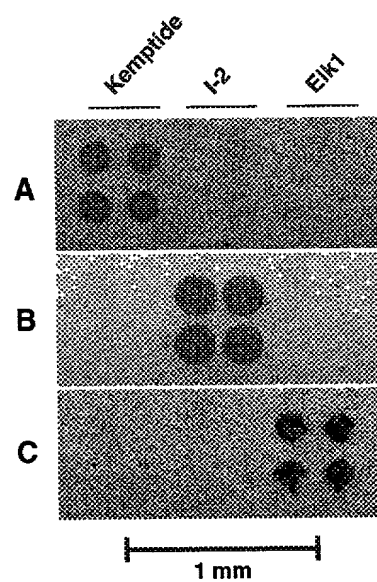


Fig. 3. Detecting the substrates of protein kinases on glass slides. **(A)** Slide incubated with the catalytic subunit of cAMP-dependent protein kinase (PKA). **(B)** Slide incubated with casein kinase II (CKII). **(C)** Slide incubated with p42 MAP kinase (Erk1).

1: A complex steroid-like molecule with a carboxylic acid side chain, multiple methyl groups, and a fused ring system including a five-membered lactone.

2: A bicyclic molecule with a carboxylic acid side chain, a sulfur atom, and a five-membered lactone.

AP1497
3a: R =

AP1767
3b: R =

AP1780
3c: R =

Fig. 4. Compounds used for the identification of the targets of small molecules. All compounds were coupled to bovine serum albumin through their carboxylate groups (either directly or via a flexible linker).

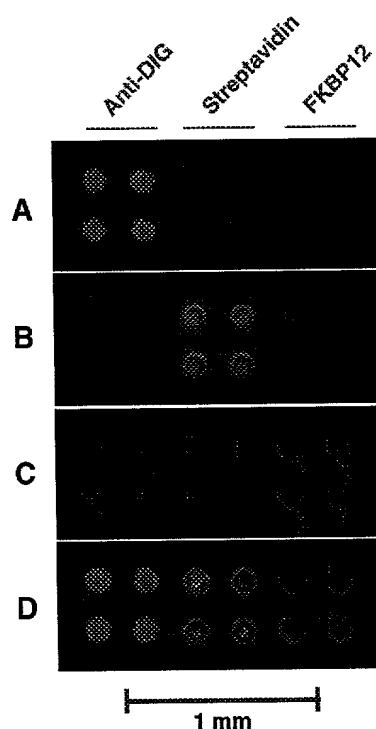


Fig. 5. Detecting the targets of small molecules on glass slides. **(A)** Slide probed with 10 ug/mL Alexa488-BSA-1. **(B)** Slide probed with 10 ug/mL Cy5-BSA-2. **(C)** Slide probed with 10 ug/mL Cy3-BSA-3a. **(D)** Slide probed with 10 ug/mL Alexa488-BSA-1 + 10 ug/mL Cy5-BSA-2 + 10 ug/mL Cy3-BSA-3a. In all panels, BODIPY-FL, Cy3, and Cy5 fluorescence were false-colored blue, green, and red, respectively.

Fig. 6. Fluorescence intensity scales linearly with the concentration of solution-phase protein over four orders of magnitude. FRB was spotted on aldehyde slides in triplicate at a concentration of 1 mg/ml. The slides were then probed with Cy5-FKBP12, ranging in concentration from 150 pg/ml to 20 ug/ml. All solutions contained 1 uM rapamycin.

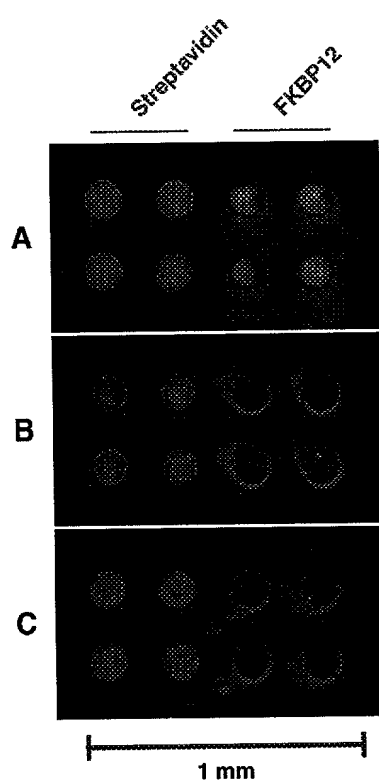
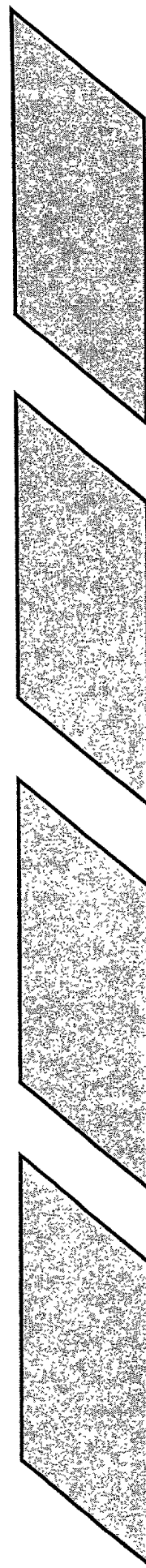


Fig. 7. Detecting the targets of low-affinity ligands on glass slides. **(A)** Slide probed with Cy5-BSA-2 + Cy3-BSA-3a. **(B)** Slide probed with Cy5-BSA-2 + Cy3-BSA-3b. **(C)** Slide probed with Cy5-BSA-2 + Cy3-BSA-3c. All conjugates were used at a concentration of 10 ug/ml. In all panels, Cy3 and Cy5 fluorescence were false-colored green and red, respectively.

FOE000" E42E2660

SCREENING METHOD 1

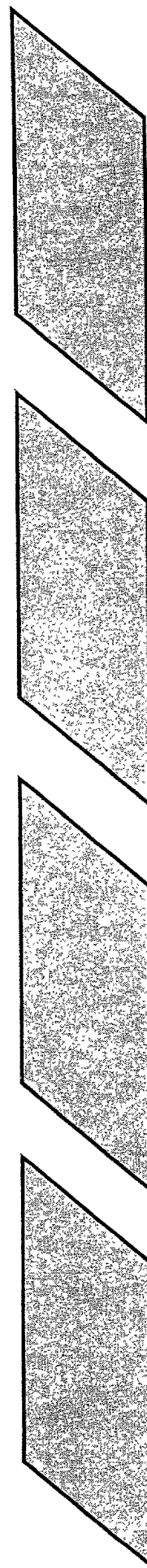
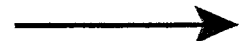
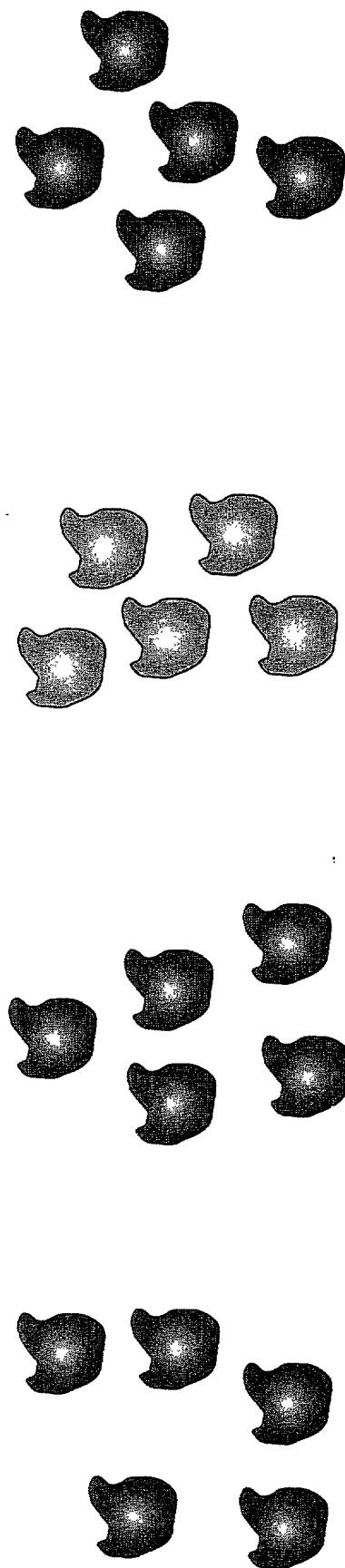
FIGURE 8A



aldehyde or BSA-NHS slides

FOE030" Et42E2560

SCREENING METHOD 1



aldehyde or BSA-NHS slides

FIGURE 8B

TOE080" E42E2660

SCREENING METHOD 1

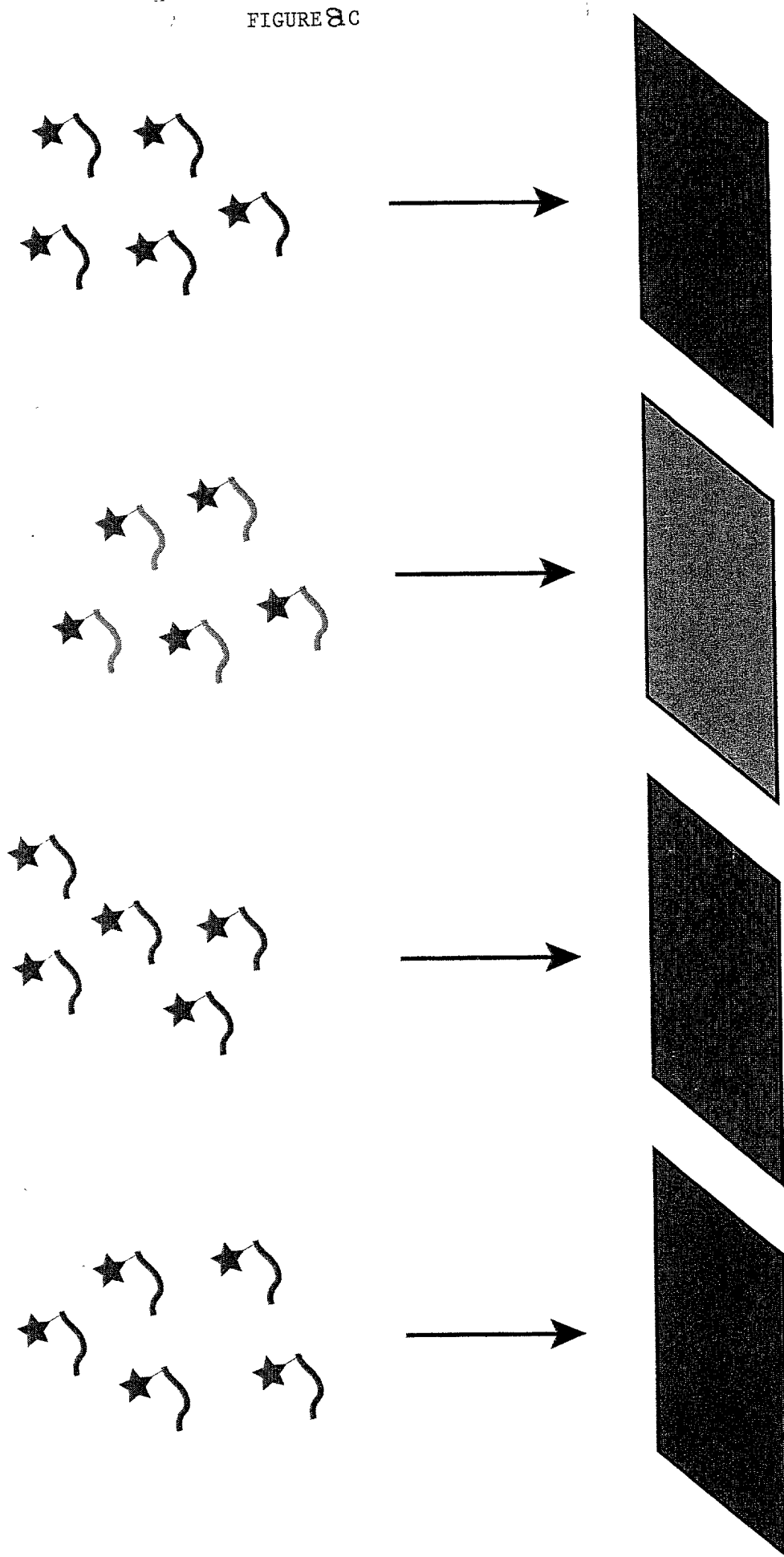


FIGURE 8C

protein slides

FOE080" E722E2560
SCREENING METHOD 1

compounds in 60% PBS / 40% glycerol

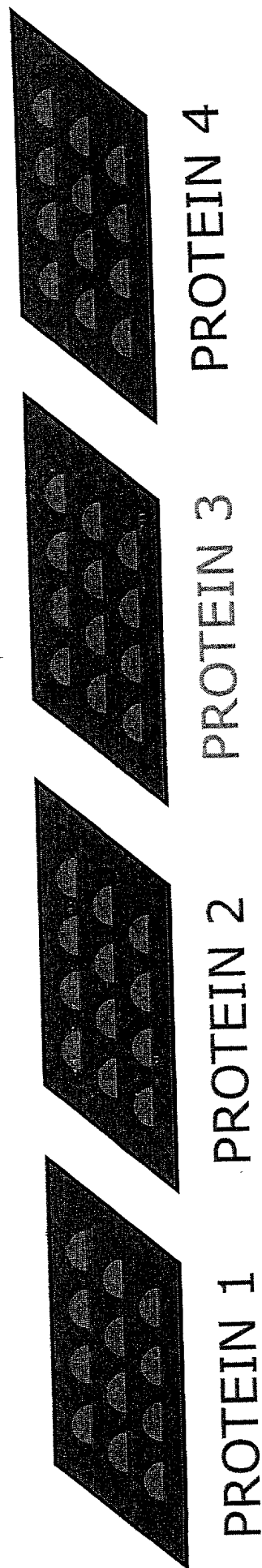
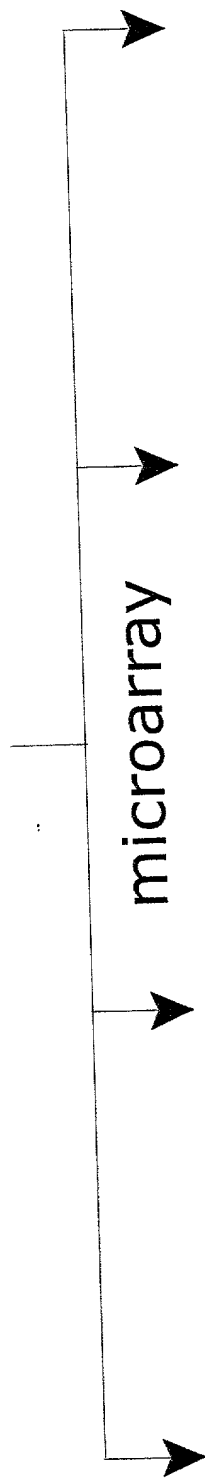
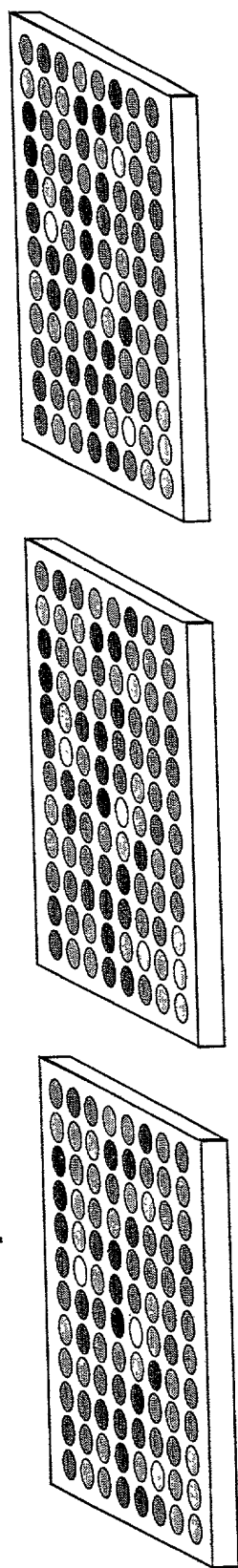
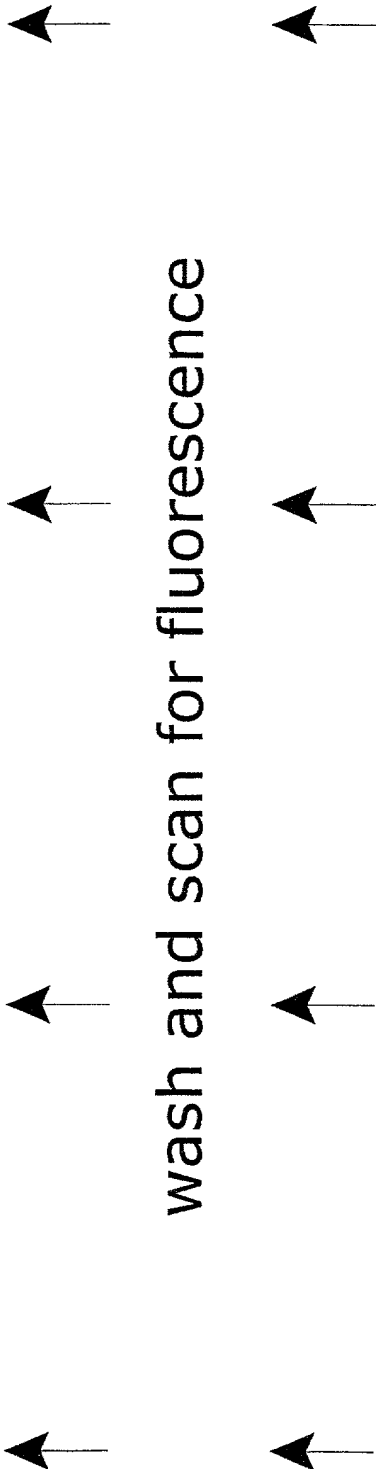
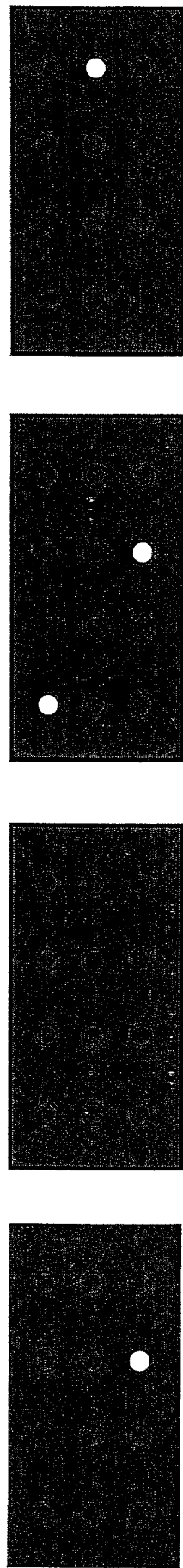


FIGURE 8D

SCREENING METHOD 1



wash and scan for fluorescence



PROTEIN 1 PROTEIN 2 PROTEIN 3 PROTEIN 4

TOE080" E42E2660

SCREENING METHOD 1

On slide
 "5-helix"
 (a domain of gp120)

Peptide ligands
 C37: 100 pM
 DCC1: 500 pM
 DCC2: 4000 nM

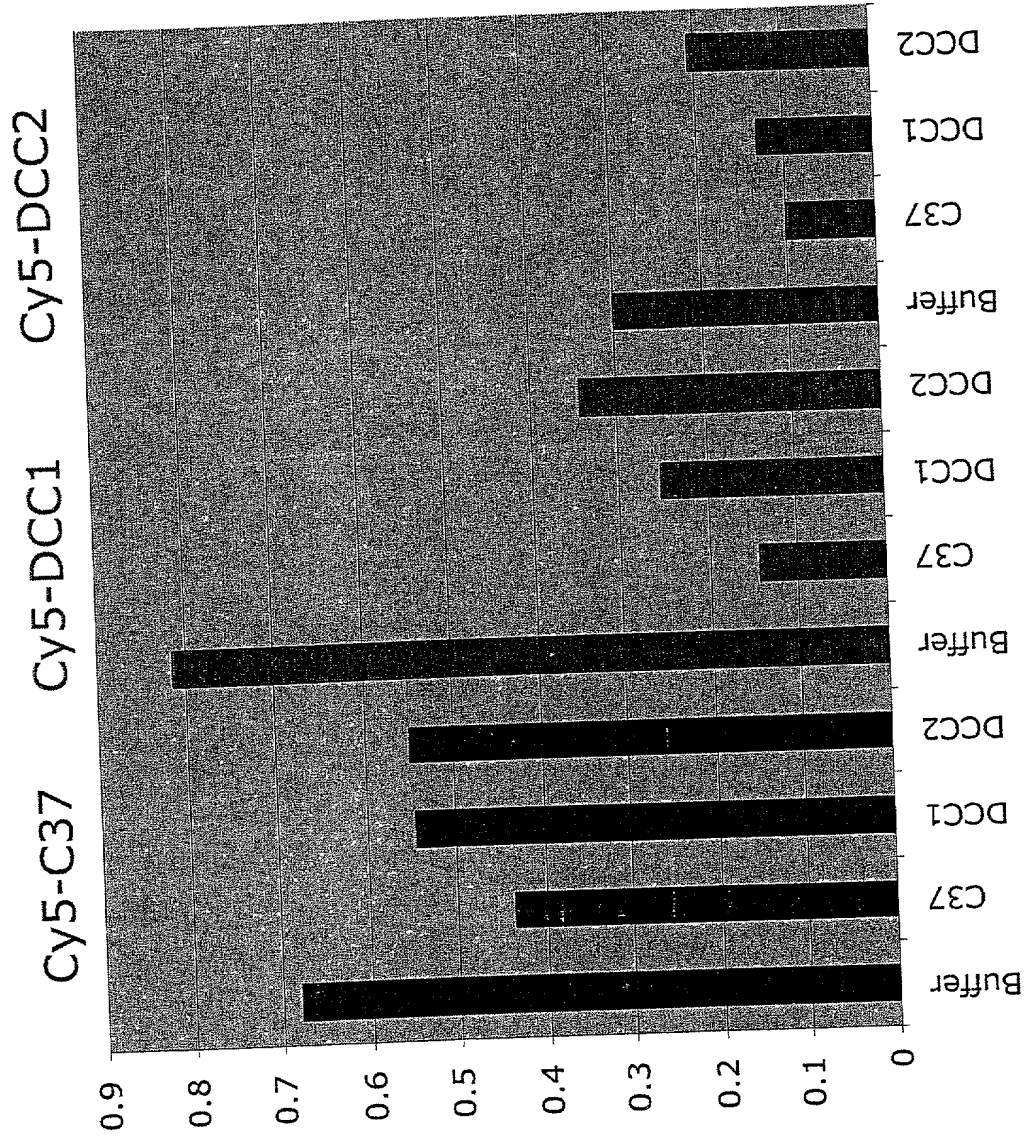


FIGURE 2